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# NEW APPARATUS AND TECHNIQUE FOR INFLATING LARVAE

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The technique described herein is a radical departure from the older methods of inflating larvae. It was designed chiefly to avoid the distortion of the anal region brought about by the use of metal clips, as well as to provide a piece of apparatus which is easily and cheaply constructed and simple to operate. It can be assembled from materials usually found in most laboratories. The principle made use of is that of the expansion of a gas within a closed membrane when exposed to decreasing outside surface pressure.

As shown in figure 1, there are but few parts. O is the oven, made of an old metal box and containing a half inch of sand, which distributes the heat more evenly. T is a large test tube which serves both as a vacuum and a baking chamber. C is a valve connecting D, the water trap, with the outside air. The trap is to prevent water from backing up into the baking tube, should a mistake occur in manipulating the valves. The vacuum is furnished by means of an aspirator or water pump (F), which is shut off from the trap by means of a clamp (E). The two pieces of rubber tubing shown are pressure tubing, the ordinary thin-walled variety being inadequate. The valve assembly and water trap are shown in detail in figure 3. A 3-hole rubber stopper holds the connecting glass tubes. Glass tubing of 4 mm bore is used. The ends of each length are held in a flame until only a very fine aperture remains. This is done to reduce the velocity of air movement in the vacuum and drying chamber. The other necessary accessory, a blowing tube, is shown in figure 4; it consists of a piece of flexible rubber tubing (B) some 8 inches long, having a mouthpiece of glass tubing in one end and a micro-pipette in the other end.

In operation, the apparatus is connected to a water tap, and a heat source is placed under the oven, care being taken not to allow the oven to become too hot. The larva, which has previously been killed in a cyanide jar, is rolled very carefully, so that the intestine is extruded and the body contents are pressed out through it. As the larval skin is emptied it is best to leave a small amount of the body fluid in the intestine, thus keeping it distended and making the next step easier. The mouthpiece of the blowing tube (fig. 4, C) is placed in the operator's mouth, and the point of the capillary (fig. 4, A) is inserted into the open end of the intestine, or, if this cannot be done, through one side. Air is then blown into the larva until it resumes somewhat its normal shape. The capillary is withdrawn, and the

still moist intestine, if pinched slightly, seals over the opening, retaining the air within the body. A looped thread is then slipped over the intestine and drawn tight a short distance from the anal opening, as shown in figure 2.

The larval skin thus prepared is dropped into the test tube (fig. 1, T) which is both sealed and connected to the water trap (fig. 1, D) by means of a rubber stopper. Valve C is closed, and the water is turned on. Valve E is then opened, and as the air in T is exhausted the air within the larval skin expands, inflating it. The amount of expansion may be controlled by regulating the flow of water or the amount of air removed from T; the larva will retain a more nearly lifelike appearance if the skin is not stretched too much.

The test tube is now placed in the oven and the larval skin thoroughly dried. This should take but a few minutes in the case of thin-walled larvae, and slightly longer if a considerable amount of tissue is present. It should be remembered that the expansion of the larva is added to when the air within it is heated. When the larva is fully baked (this can best be determined by experience), valve E is closed and the flow of water shut off. Then valve C is opened very carefully so as to allow the pressure in the test tube to be gradually equalized with that of the atmosphere. If valve C is opened too suddenly, the dried skin will collapse. Should this happen, it is possible to expand the larva again, by once more creating a vacuum in the tube.

The larva is then removed from the baking chamber, and the intestine is pinched off close to the body wall with a pair of forceps. Since it is very thin and brittle, the intestine breaks off readily, leaving a sizable opening for mounting the larva on a wire, if so desired.

The type of valve illustrated in figures 1 and 3 has been shown chiefly because it is the cheapest, and perhaps the easiest to assemble from materials at hand. However, it is not the best valve for the purpose. A single or a 3-way glass stopcock is superior, since the flow of air into the drying chamber can be better regulated. The most satisfactory results may be obtained from a 3-way stopcock.

As shown in figure 5, both the apparatus and the method of operation are somewhat simplified by the use of a 3-way stopcock. Valve E (fig. 1) is eliminated. The stopcock is manipulated as follows (fig. 5): First, D is shut off from the outside air and connected with A. After the larva is dried, D is shut off from A, and A is connected with the outside air. This equalizes the pressure in A and eliminates any back-flow of water when the aspirator is turned off. Then D is connected with the outside air.

In case the laboratory is not equipped with an aspirator or running water, a sufficient vacuum might be obtained by using a siphon system, employing two 5-gallon jars. This has not been tried, but is mentioned as a possibility.

There are several advantages in using this technique rather than the clip and air-pressure method. As previously mentioned, the anal region is not

distorted. Then, too, there is no danger of the larva being blown into the oven and destroyed, as often happens when a clip is employed. A more even distribution of heat is obtained, and a high temperature is not necessary, so that burning the larva is not likely. It is less difficult to blow air into the larva through a micro-capillary than to attempt to fix a larval skin on the end of a pipette by means of a clip. Particularly is this true in the case of medium and smaller sized larvae, to which this technique is especially applicable. Further, the annoying tendency of the larval skin to adhere to the inflating tip is not met with. Attempts to remove a larva from the tip under these circumstances have often resulted in the breaking off of the anal prolegs, or the entire anal region. More lifelike specimens can be prepared, since the distension of the larval skin can be better controlled than when direct air pressure is used. Finally, the apparatus is small and compact, and can be constructed at a low cost.

## Explanation of Illustrations

Figure 1.--Apparatus in position for use. O, oven; T, test tube which serves both as vacuum and drying chamber; C, valve to outside air; D, water trap; E, clamp; F, aspirator or water pump.

Figure 2.--Dried larva, showing thread tied about the everted intestine.

Figure 3.--Details of water trap and connections. A, pressure tubing to aspirator; B, pressure tubing to vacuum chamber; C, valve to outside air; D, water trap; E, clamp shutting aspirator off from water trap.

Figure 4.--Details of blowing tube. A, micro-pipette; B, rubber tubing; C, mouthpiece of glass tubing.

Figure 5.--Details of water trap and 3-way stopcock. A, pressure tubing to aspirator; B, pressure tubing to vacuum chamber; C, 3-way stopcock; D, water trap.

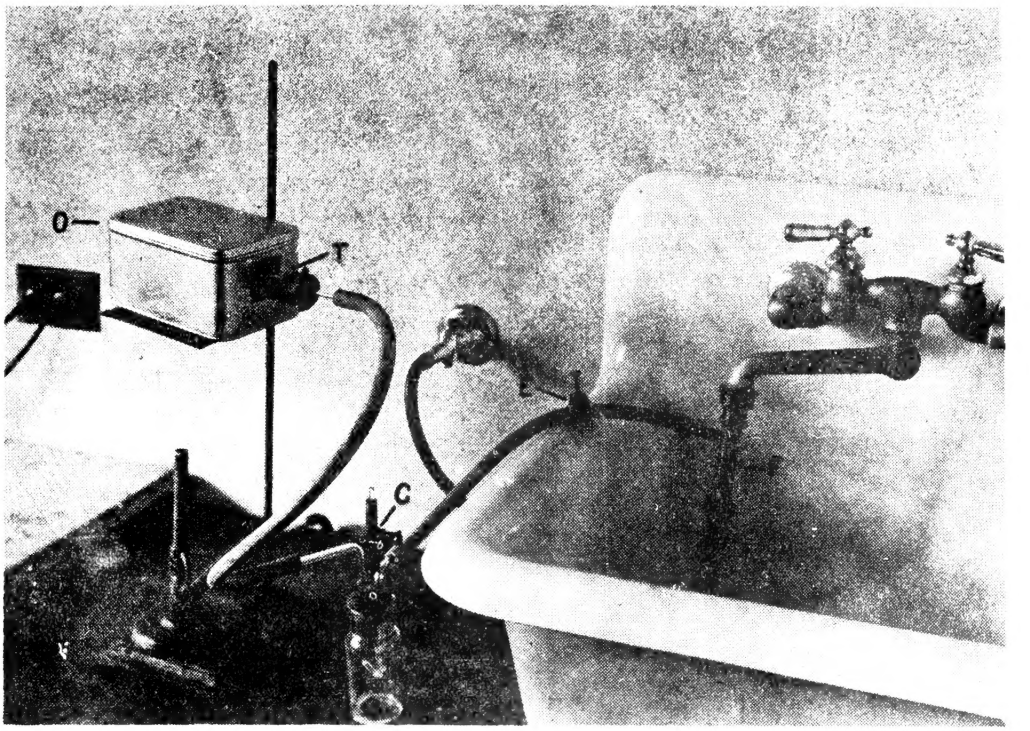


Fig. 1

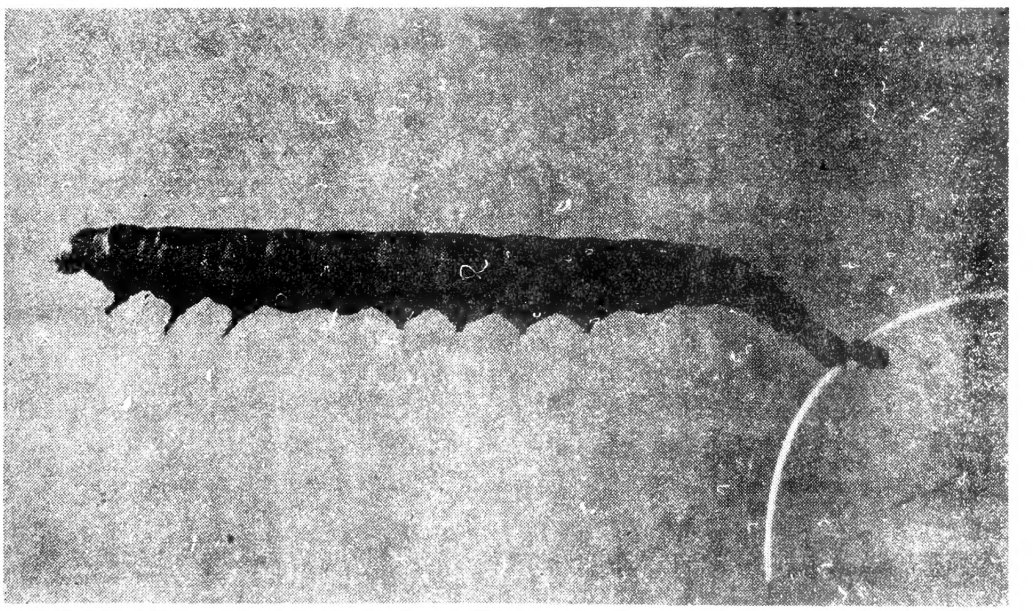

Fig. 2

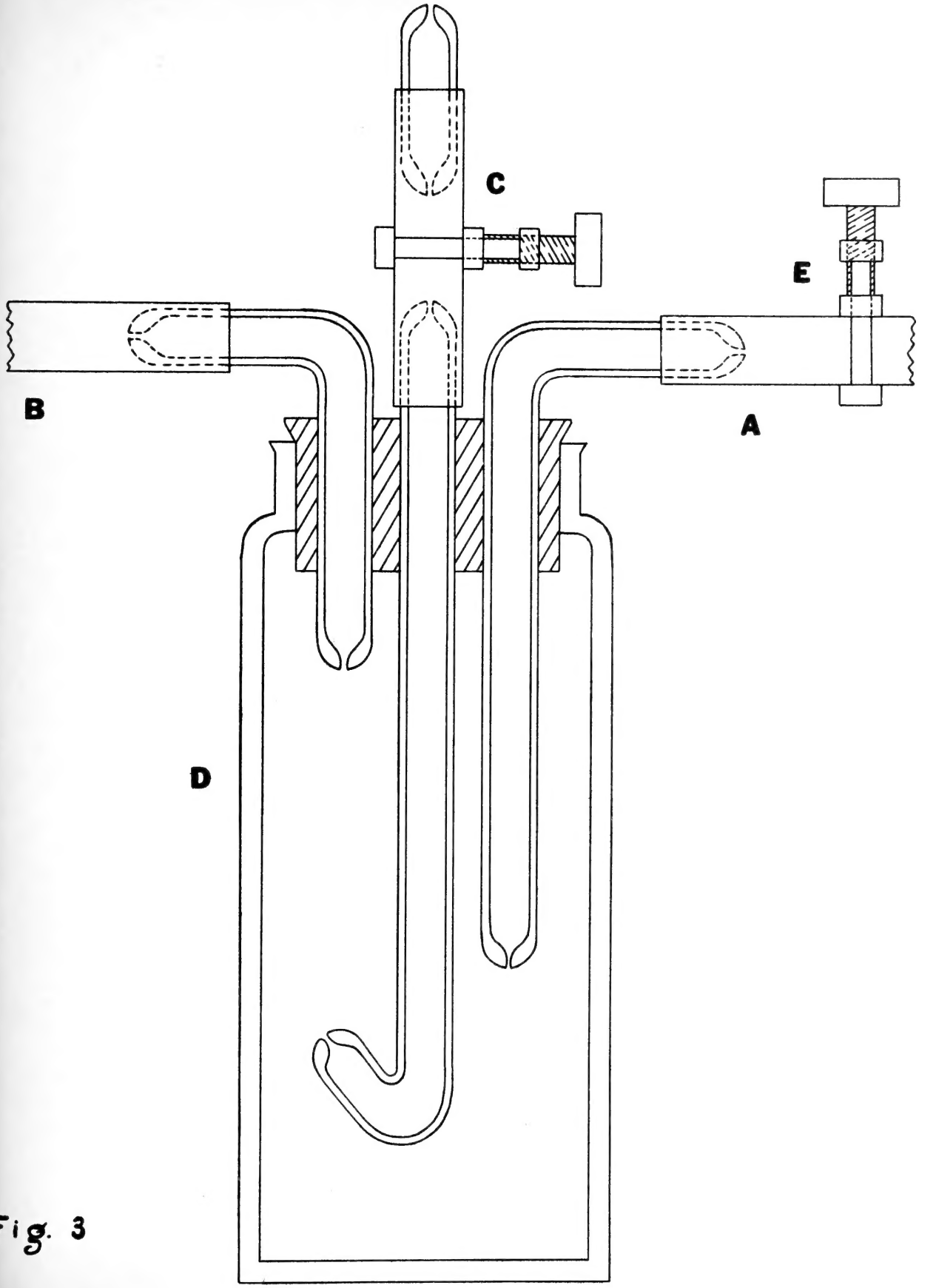


Fig. 3

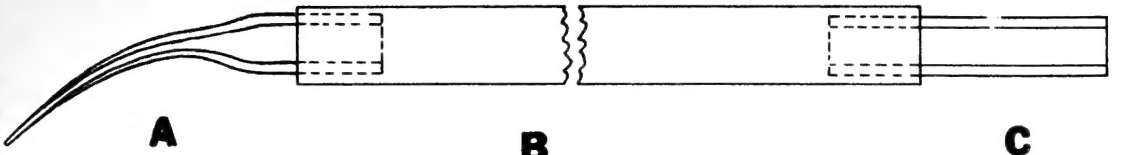


Fig. 4

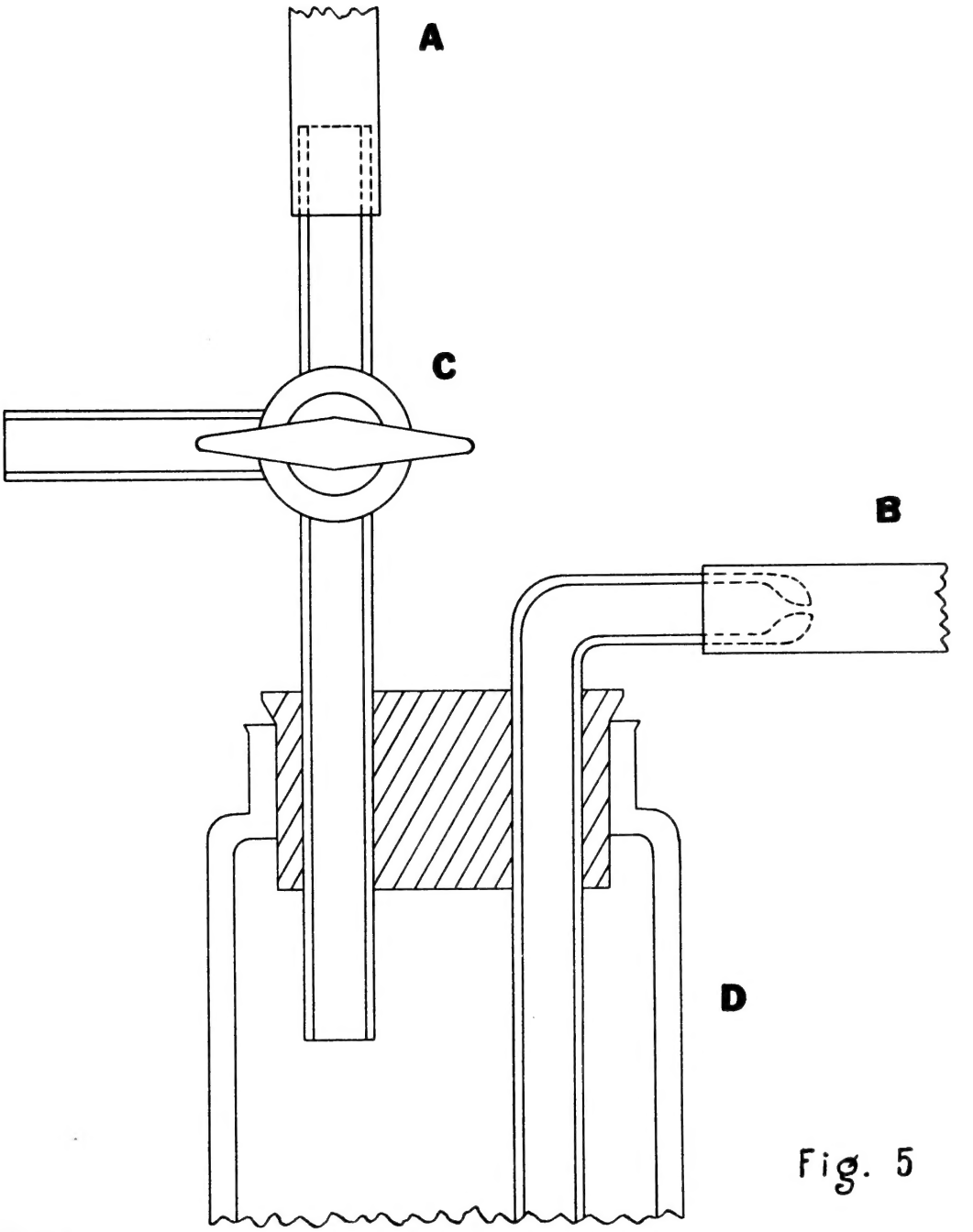


Fig. 5